Implications of Diffusion-Controlled Limit for Processivity of Dimeric Kinesin Head Domains

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ABSTRACT The diffusion-limited rate for association of the ADP complex of dimeric DKH392 kinesin head domains with a microtubule was estimated to be 2–3 $\times$ 10^7 M^{-1} s^{-1} based on approximation of a microtubule as a highly elongated prolate ellipsoidal adsorber of 100% efficiency. This theoretical bimolecular rate is ~100-fold smaller than the experimental rate, $k_{cat}/K_{0.5}^{MT}$, for DKH392 that was determined from the stimulation of the steady-state ATPase rate by microtubules. The large difference between these two estimates of the bimolecular rate indicates that it is likely that dimeric DKH392 hydrolyzes multiple ATP molecules during each diffusional encounter with a microtubule.

INTRODUCTION

Kinesin has the unique property among molecular motors in that a single molecule can remain attached to a microtubule (MT) while driving the net sliding of the MT (Howard et al., 1989). Such single motor motility requires that a molecule of kinesin can prevent diffusional escape of a MT even though the kinesin must, at least transiently, release from the MT to relocate to a different position. This property of in vitro motility assays has been in striking contrast to the biochemical properties of native kinesin as measured in solution with a low rate of MT-stimulated hydrolysis and weak binding to MTs in the presence of ATP (see Huang and Hackney, 1994).

Recently, a kinesin preparation has been reported that has the biochemical properties in solution expected for a motor capable of single motor motility (Huang et al., 1994; Hackney, 1994a). This construct is designated DKH392, and it consists of the first 392 amino acids of *Drosophila* kinesin heavy chain (Yang et al., 1989) expressed in *Escherichia coli*. This protein is dimeric and has a high maximum ATPase rate in the presence of MTs (k_{cat} = ~40 s^{-1}) and tight binding to MTs in the presence of ATP ($K_{0.5}^{MT}$ < 50 nM MTs expressed as the concentration of tubulin $\alpha\beta$ dimers). As with native kinesin, release of ADP is slow in the absence of MTs and binding of the DKH392 · ADP complex to MTs accelerates ADP release. Interestingly, DKH392 exhibits half-site reactivity in which a dimer of DKH392 releases only half of its bound ADP on association with a MT (Hackney, 1994b).

If DKH392 hydrolyzes only one molecule during each diffusional encounter with a MT, then the bimolecular rate for diffusional association of the DKH392 · ADP complex with MTs must be at lease as great as $k_{cat}/K_{0.5}^{MT}$, which approaches 3 $\times$ 10^9 M^{-1} s^{-1} at low occupancy of the MT lattice by head domains (Hackney, 1994a). This apparent encounter frequency for DKH392 with tubulin subunits of a MT approaches the maximum diffusion-controlled encounter frequency in water of 7 $\times$ 10^9 M^{-1} s^{-1} for two spheres of equal size (see Fersht, 1984). Diffusional encounter of kinesin head domains with the tubulin subunits of a MT would be expected to be considerably slower than this maximum value, however, because diffusional approach of a head to a single tubulin dimer of a MT is restricted by the other tubulin subunits of the MT and because many tubulin dimers have to compete for the binding of each kinesin that approaches the MT. As discussed previously (Hackney, 1994a), this possible violation of the diffusion-controlled limit for DKH392 would be eliminated if DKH392 were processive with hydrolysis of multiple molecules per diffusional encounter with a MT. This communication now presents a theoretical analysis of the diffusion-controlled limit for association of DKH392 with the individual tubulin dimers of a MT. The diffusion-controlled limit determined in this way is 100- fold smaller than $k_{cat}/K_{0.5}^{MT}$ and, consequently, DKH392 likely hydrolyzes many molecules per diffusional encounter with a MT.

THEORY

Because of the large size and high axial ratio of a MT, it can be approximated as a stationary prolate ellipsoid with a large ratio a/b of the major to minor axes. For a highly elongated ellipsoidal adsorber of 100% efficiency in an infinite medium of concentration C_0, Berg (1983) gives the inward flux I for capture of a diffusing component by the adsorber at steady state as $I = 4pD_{20,w}aC_0/\ln(2a/b)$, where $D_{20,w}$ is the diffusion coefficient of the diffusing component. For a MT, the relevant minor axis b will be given by half of the diameter of the MT plus half of the diameter of an attached kinesin. This larger surface represents the closest approach that is possible for the center of mass of the kinesin head to the MT. The diameter of the MT is approximately 24 nm, and a kinesin will be assumed to have a diameter of 4 nm giving a value of 14 nm for b. For initial calculation, a length of 20 mm for the MT will be assumed, which corresponds to a value of 10 mm for a. For a $D_{20,w}$ of DKH392 of 4.9 $\times$ 10^7 cm^2/s (Huang

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et al., 1994) and a C_0 of 1 M expressed in units of mol/cm^3, the calculated inward flux is 5.1×10^{11} s^{-1} expressed as the number of molecules of the diffusing component captured by each MT per second. Because a 20-mm MT with 13 protofilaments and a spacing of 8 nm per tubulin heterodimer along each protofilament contains 3250 tubulin dimers, this corresponds to a capture rate per tubulin dimer of 1.6×10^7 s^{-1}. The bimolecular association rate for kinesin with tubulin dimers, k_{bi}, will correspondingly be 1.6×10^7 M^{-1} s^{-1} because a C_0 value of 1 M was used in the calculation and the $D_{20,w}$ of the MT is too small to contribute significantly.

This analysis is subject to a number of qualifications. The calculation was performed with a $D_{20,w}$ value for DKH392 in pure water at 20°C, whereas the kinetic experiments were performed in buffered solution at 25°C. The diffusion-limited k_{bi} value in water at 25°C would be larger by 12.5% because of decreased viscosity, but this would be partially offset by the increase in viscosity produced by the buffers and salts that are present in the kinetic measurements. In regard to the approximation of a MT by an elongated prolate ellipsoid, a cylinder of the same dimensions would be a more appropriate model and would have a surface area that is 27% larger. The capture rate would be expected to increase by a much smaller factor, however, because it is proportional roughly to length rather than area (see Berg (1983) and below for influence on increased b) and because kinesin heads cannot attach to the terminal faces of a cylindrical MT, whereas all of the surface of the ellipsoid is assumed to be adsorptive. An additional complication is the effective diameter of the kinesin used to estimate b, but the calculations are highly insensitive to the exact value used for the diameter of kinesin because b is dominated by the diameter of the MT and because b only appears in a logarithmic term. Thus, an increase from 4 to 8 nm for the diameter of the kinesin head increases b from 14 to 16 nm (14% increase) with an approximately proportional increase in surface area, but only increases the theoretical k_{bi} value by 2%.

Although the above calculation was performed on a MT of 10 mm in length, the value of k_{bi} is not highly sensitive to the length of the MT. For a 10-fold shorter MT of 2 mm, the theoretical k_{bi} only increases 70% to 2.7×10^7 M^{-1} s^{-1}. Thus, for MTs of the lengths usually obtained by polymerization of purified tubulin and stabilized by taxol, a value of $2–3 \times 10^7$ M^{-1} s^{-1} would be a reasonable upper limit for the diffusion-controlled rate of encounter of DKH392 with the tubulin dimers of a MT. For monomeric DKH340, the corresponding estimate would be 60% higher because of the larger $D_{20,w}$ value of DKH340.

DISCUSSION

The maximum encounter frequency of DKH392 with tubulin subunits of a MT of only $2–3 \times 10^7$ M^{-1} s^{-1} is over 100-fold smaller than the kinetically derived $k_{cat}/K_{0.5}^{MT}$ value of $\sim 3 \times 10^9$ M^{-1} s^{-1} on a per dimer basis. This discrepancy will be even larger for productive encounters of a kinesin · ADP complex with a MT leading to release of ADP. The bimolecular rate for such productive encounters is likely to be slower than the theoretical diffusion-controlled limit because not all encounters of DKH392 with a MT will be productive because of incorrect orientation or lack of sufficient activation energy for the reactions leading to ADP release. This discrepancy can be eliminated if the assumption of only one ATP hydrolyzed per diffusional encounter is not valid. Thus, a minimum of 100 molecules are likely to be hydrolyzed by DKH392 during each diffusional encounter with a MT, and the actual number per productive encounter may be considerably higher. This is precisely the behavior required of a processive motor needed to produce single motor motility. Models for how such processivity could be produced by a dimeric structure have been proposed (Hackney, 1994b) that are consistent with the known kinetic properties of DKH392. Recent investigation of the stimulation by MTs of the release of fluorescent mant-ADP from DKH392 has indicated that the experiment k_{bi} value for productive encounter is in fact in the range of $1–2 \times 10^7$ M^{-1} s^{-1} (our unpublished data).

By this criteria, monomeric DKH340 with a $k_{cat}/K_{0.5}^{MT}$ of 5×10^8 M^{-1} s^{-1} (Huang and Hackney, 1994) hydrolyzes 10–15 molecules per diffusional encounter with a MT and possibly more per productive encounter. Hydrolysis of multiple molecules in this case may represent rapid recapture of a DKH340 · ADP complex by the MT or the failure of DKH340 to release even transiently from a tubulin dimer during each cycle of hydrolysis. It is not currently possible to estimate the probability of recapture by the MT because the length of any refractory period is unknown (e.g., the time between ATP-induced release of DKH340 from tubulin and the transition of the DKH340 · ADP complex to a form that is competent for rebinding to the MT). In this regard, recent work has indicated that monomeric kinesin head domains that are longer than DKH340 have high k_{cat} values, but much weaker binding to MTs in the presence of ATP (our unpublished observations). These longer monomers apparently cycle more effectively on and off the MT during hydrolysis of each and thus may be more appropriate models for a monomer head domain operating in a distributive manner.

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DISCUSSION

Session Chairperson: Margaret A. Titus
Scribe: Alexander L. Friedman

JOHNSON: We have other direct evidence for showing that kind of processivity that we're not quite ready to share. But the fundamental question is the underlying basis for that processivity. And I've always felt that this observation of one head binding and the other hanging off in solution is contrary to what you might expect for a processivity where you would require if anything you should have two heads attached and rarely only one attached. It seems like a bit of a contortion to get it to fit into a model of hand-over-hand movement. The other question is, you've done some experiments on the mant-ADP measurements of release, and the question is whether you've extended those measurements into a time resolution sufficient to be able to ask whether you're getting half of the heads coming off after the ADP is coming off fast and half coming off slow.

HACKNEY: The short answer to that is "no we have not" tried to do those experiments yet, but we plan to.

JOHNSON: The other question is, it seems that the processivity in fact is very ionic strength-dependent as a function of the rebinding rate. Some of the differences that we see, I think, are a function of the differences in the rate of kinesin binding as a function of ionic strength. In particular I want to remind you, in the early studies using ATP-induced association as an affinity purification tool, that's always with ATP and salt being added. That shifts the equilibrium toward dissociation.

HACKNEY: That's also the fact that it folds up, and we think that's part of why it doesn't work.

JOHNSON: Yeah.

HACKNEY: If you stop and think about it, that's the wrong way for a motor to work. It shouldn't fall off when you add ATP. It falls off because it's in this folded form, which is designed not to work. So in fact if there were really active motors lurking around you wouldn't find them that way because they may stay bound after you add the ATP.

JOHNSON: Yeah. The final question is, it seems that processivity is apparent at lower salt concentrations and the real question is, to what extent processivity of a small molecule bound to a microtubule by itself continues into the physiological ionic strength conditions of 150 mM salt.

HACKNEY: I think it's going to be very much less. These experiments are done roughly at, I believe, 48 mM as the ionic strength. So it's not physiological, but it's not one of the really low ionic strength buffers. And if I can comment on his first comment, I would say in fact that I find these types of models to be just what one would like. I think they're nice because they solve two thing[s] a motor has to do. If the heads are both bound and one has to move, it is in many cases going to be trying to pull the attached head. That represents a load on the head that's going to move. The other thing is there's likely to be some sort of refractory phase after a head is released before it can bind back to the microtubule. And this model leaves this head there long enough for it to go through this refractory phase and to be primed and ready to add back when it's allowed to add back by the ATP binding to the attached head.

JOHNSON: I hope we're not going to resurrect the Eisenberg-Taylor controversy on refractory states.

WONG: I have a question about your calculation of the maximum collision rate between a microtubule and kinesin in your abstract. If I understood this correctly, the equation you used was based on assuming the microtubule was a perfect absorber for kinesin.

HACKNEY: Yes.

WONG: OK. If that's true, that means that every collision that kinesin makes with a microtubule would cause the disappearance of kinesin. That would create a depletion zone next to the microtubule, and that would definitely make your calculation of the maximum collision rate lower than what really happens if it were a diffusion-limited reaction.

HACKNEY: There will be a zone where there will be a lower concentration but, presumably, it comes off at some rate too. The calculation was just to give the absolute maximal rate that it might bind. The real rate is thought to be lower than that. Also, these rates are highly dependent on the salt concentration.

WONG: Definitely. I did a rough calculation assuming it's not a perfect absorber, just to calculate the collision rate. Of course, it's very rough. I think it's right on the order of magnitude. The number comes out 600 times higher than what you estimated.

HACKNEY: I would have to look at those calculations before I could comment on them. One thing you have to realize: the rate that the head binds to this large microtubule is going